ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.91

Volume 130, pp. 91–103 *January–March 2015*

Cladonia corymbescens consists of two species

Teuvo Ahti, Raquel Pino-Bodas, & Soili Stenroos

*Botanical Museum, Finnish Museum of Natural History, University of Helsinki,
P.O. Box 7, FI-00014 Helsinki, Finland*

* Correspondence to: *teuvo.ahti@helsinki.fi*

Abstract — The lichen *Cladonia corymbescens* (*Cladoniaceae*, *Ascomycota*) has been reported to have two main ranges, one in Melanesia and Australasia and another in the Himalayas and surrounding areas. When specimens from New Caledonia, Bhutan, and Thailand were subjected to molecular sequencing (ITS rDNA and RPB2), two distinct clades were detected. The clades were also supported by morphological and geographical differences supporting recognition of two distinct species. *Cladonia corymbescens* s. str. is present in the Philippines, Melanesia, and Australasia, while the South East Asian mainland material is referred to *C. pseudofissa*, a new combination at species level.

Key words — *Cladonia furcata* group, lichens, molecular systematic, phylogeny, taxonomy

Introduction

One of the most common fruticose species of *Cladonia* in the mountains of western Melanesia is *Cladonia corymbescens*. The nomenclature of this species and its similarities with other species was thoroughly discussed by Stenroos (1988), who also reported it from China and Nepal. However, Abbayes (1974) was the first lichenologist to recognize this species from the Himalayas. His opinion was also accepted by Awasthi & Ahti (2007) and Rai et al. (2014), who stated that the species is widespread in the mountains in India and Nepal. In his world monograph, Vainio (1887) could not give a definite opinion on the type material of *C. corymbescens* from New Caledonia, and he referred the only Himalayan specimen representing that species that he apparently examined to *C. erythrosperma* var. *thomsonii*.

Several studies using molecular data have shown that many morphological *Cladonia* species are not monophyletic (Kotelko & Piercey-Normore 2010, Fontaine et al. 2011, Pino-Bodas et al. 2011, Steinová et al. 2013). The re-examination of the morphology based on molecular data in lichenized fungi has revealed that in several cases some phenotypical differences have been overlooked (Argüello et al. 2007, Núñez-Zapata et al. 2010, Parnmen et al.

2013, Muggia et al. 2014). The aim of this study was to use DNA sequence data to assess whether *Cladonia corymbescens* is a genetically homogeneous species and to revise its classification based on the phylogenetic results and morphological differences.

Materials & methods

Material for DNA analyses

Altogether 39 specimens of 29 species of *Cladonia* representing the supergroup *Cladonia* in the provisional classification of Stenroos et al. (2002) were selected for a phylogenetic analysis in this study. These specimens are preserved in the herbaria of University of Helsinki (H), University of Turku (TUR) or Complutense University, Biology Faculty (MACB); details of the collections are shown in Table 1. Four specimens of *Cladonia corymbescens*, two from New Caledonia and two from the Himalayas, were available for DNA analyses, while 60 sequences had been generated in previous studies (Stenroos et al. 2002; Pino-Bodas et al. 2010a, 2010b, 2012a, 2012b, 2012c, 2013a, 2013b). *Cladonia rangiformis* Hoffm. was selected as outgroup based on previous results (Stenroos et al. 2002).

DNA extraction, PCR, and sequencing

Total DNA was extracted using DNeasy Plant Mini Kit (QUIAGEN, Germany) following the manufacturer's instructions. In this study two genetic regions were chosen: internal transcribed spacer of ribosomal DNA (ITS rDNA) and the second largest subunit of RNA polymerase II (RPB2). Although the ITS rDNA region has been chosen as standard barcoding for fungi (Schoch et al. 2012), in *Cladonia* the ITS rDNA frequently fails in the identifications (Kelly et al. 2011; Pino-Bodas et al. 2013b) and RPB2 gives better results (Pino-Bodas et al. 2013b). The primers used to amplify the nuclear ITS nrDNA were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and for RPB2 they were RPB2dRaq/RPB2rRaq (Pino-Bodas et al. 2010a) or CLRPB25F/CLRPB27R (Yahr et al. 2006). The amplification programs are listed in Pino-Bodas et al. (2013a). PCR was carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). Amplifications were prepared for a 25 μL final volume. PCR was performed using a Mastercycler ep Gradient S (Eppendorf, Westbury, NY). PCR products were purified with ExoSAP-IT (USB Corporation, USA) and sequenced in Macrogen Europe (Amsterdam, Netherlands).

Alignments and phylogenetic analyses

The forward and reverse strands were assembled and edited in Sequencher^{TM} v. 4.1.4 (Gene Codes Corporation, Inc., Ann Arbor, Michigan, USA). BLAST searches were used to confirm that the obtained sequences belonged to *Cladonia*. The sequences were manually aligned in BIOEDIT (Hall 1999). Nine ambiguous positions were manually delimited and removed from the ITS rDNA alignment. The RPB2 alignment did not have ambiguous positions.

Every region was separately analyzed by Maximum Likelihood (ML), with 500 bootstrap replicates to assess the support of each node. The conflict between the loci was tested according Lutzoni et al. (2004). This method considers the existence of

TABLE 1. List of *Cladonia* specimens used in the molecular study with voucher specimen information and GenBank accession numbers.
(The new sequences and the new combination are in bold font.)

SPECIES	COLLECTION	CODE	ITS rDNA	RPB2
C. acuminata	USA, Ahti 63278 (H)	1ACUMI	JN621932	JN621965
C. apodocarpa	USA, Harris 54250 (H)	1APODO	KC526127	KC526068
C. borbonica	New Caledonia, Dennetière 109 (H)	AT686	AF455214	**KP732364**
C. cariosa	Spain, Burgaz (MACB 94208)	5CARI	JN621909	JN621941
C. cartilaginea	Brazil, Stenroos 4926 (TUR)	LK45	AF455212	—
C. ceratophylla	Brazil, Stenroos 5081 (TUR)	LK37	AF455171	—
C. conista	Spain, Burgaz (MACB 92796)	2HUMIL	JF926613	JF926567
	Russia, Zolotuchin 26a (H)	1CONIST	JF926633	JF926568
C. corsicana	Spain, Burgaz (MACB 100763)	SP1	JF288797	JF288833
	Spain, Burgaz (MACB 101074)	SP2	JF288798	JF288834
C. corymbescens	New Caledonia, Dennetière 45 (TUR)	AT680	AF455235	**KP732366**
	New Caledonia, Christenhusz 6169d (H)	CL166	**KP732363**	**KP732367**
C. cyathomorpha	Spain, Burgaz (MACB)	1CYATH	KC415941	KC525275
	Norway, Ahti 68660 & Tønsberg (H)	3CYATH	KC415943	KC525276
C. firma	Spain, Burgaz (MACB 91619)	1FIRM	FM205907	FM207568
	Spain, Burgaz (MACB 90655)	7FIRM	FM205910	FM207576
C. foliacea	Portugal, Burgaz (MACB 90506)	1FOL	FM205894	FM207569
C. furcata	USA, Ahti 58283 (TUR)	AT638	AF455220	**KP732369**
C. humilis	Spain, Burgaz (MACB 95931)	9HUMIL	JF926615	JF926576
	Croatia, Burgaz (MACB 101103)	20HUMIL	JF926621	JF926580
C. multiformis	Canada, Ahti 57065 (H)	LK70	AF455213	**KP732370**
C. nana	Brazil, Stenroos 4940 (TUR)	LK34	AF455211	—
C. neozelandica	New Zealand, Wirth 28180 (TUR)	AT590	AF455206	—
C. petrophila	USA, Ahti 56654 (H)	LK68	AF455222	—
C. peziziformis	USA, Stenroos 5198 (TUR)	AT631	AF455221	—
	USA, Ahti 56670 (H)	LK38	AF455182	—
C. pityrophylla	Brazil, Stenroos 5096 (TUR)	LK40	AF455238	—
C. pseudofissa	Thailand, Parnmen 285 (H)	1CORYMB	**KP732362**	**KP732365**
	Bhutan, Søchting 9206 (H)	LK35	AF455239	**KP732368**
C. pulvinata	Spain, Burgaz (MACB 91646)	4PUL	FM205911	FM207579
C. pulvinella	USA, Ahti 69191 (H)	CLCAL4	KC415963	KC525287
C. pyxidata	Greenland, Hansen (H)	17PYXI	KC415983	KC525269
C. rangiformis	Netherlands, Van der Goes et al. (H)	5RANG	JN811400	JN811429
	Sweden, Skytén (H)	6RANG	JN811401	JN811430
C. scabriuscula	Canada, Ahti 56969 (H)	LK11	AF455217	**KP732371**
C. subcariosa	USA, Moore (H)	1SUBCARI	JN621936	JN621969
C. subconistea	Korea, Moon 7188 (H)	1SUBCONI	KC415949	KC525296
C. subulata	Spain, Burgaz (MACB 93151)	1SUBU	FN86566	HM243210
C. turgida	Canada, Lendemer (H)	1TURG	JF288801	KC526089

incongruity whenever a clade was supported with a bootstrap of more than 75% in a locus, while in other loci the individual sequences of this clade were part of another clade with bootstrap support ≥75%. The trees were checked manually. No conflicts were detected and the datasets were combined. The combined dataset was analyzed by Maximum Parsimony (MP), ML, and Bayesian analyses. MP analysis employed PAUP version 4.0.b.10 (Swofford 2002), using heuristic searches with 1000 random taxon-addition replicates with TBR branch swapping and MulTrees option in effect, and equally weighted characters and gaps treated as missing data. Bootstrap with 1000 replicates was used as the confidence analysis, using the heuristic option. The ML and Bayesian analyses were run considering four partitions (ITS rDNA and each codon position of RPB2). Maximum Likelihood analyses (ML) were done in RAxML 7.04 (Stamatakis 2006), assuming the model GTRGAMMA. A fast bootstrap with 500 replicates was implemented to assess the support of each node. Bayesian analysis was carried out using the program MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The models of evolution were selected with MrModeltest (Nylander 2004) under AIC criterion. The models selected were GTR+I+G for ITS rDNA and SYM+I+G for RPB2. Posterior probabilities (pp), approximated by sampling trees using Markov Chain Monte Carlo (MCMC), of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. Model parameters were estimated in each analysis for 5,000,000 generations sampled in four simultaneous chains, and every 1000th was saved into a file. When the analysis was finished we checked that standard deviation between the runs was <0.005. The program AWTY (Nylander et al. 2008) was used to determine when the chains reached the stationary stage. The first 1000 trees were deleted as the "burn in" and the 50% majority-rule consensus tree was calculated using the "sumt" command of MrBayes.

Morphology and chemistry

About 63 herbarium specimens of *Cladonia corymbescens* s. lat. deposited in Helsinki (H) were morphologically re-evaluated after the phylogenetic analyses. Authors Ahti and Stenroos had previously studied many additional specimens in various herbaria [e.g., Geneva (G), Leiden (L), Lucknow (LWG, LWG-LWU, LWG-AWAS)] as well as material borrowed from Edinburgh (E); see also the specimen lists in Stenroos (1988) and Rai et al. (2014).

The secondary metabolites were analyzed by thin layer chromatography (TLC) according to standardized procedures (Orange et al. 2001) using the solvents A (Toluene: dioxane: acetic acid, Merck) and B (Hexane: diethylether: formic acid, Merck).

Results

In this study we generated 10 new sequences (2 of ITS rDNA and 8 of RPB2). The combined dataset contained 1332 unambiguous characters (597 of ITS rDNA and 735 of RPB2), 267 of which were parsimony informative (152 of ITS rDNA and 115 of RPB2). The MP analysis generated two equally parsimonious trees of 886 steps. The ML analyses of the combined dataset generated a tree with likelihood value of –LnL = 6355.82, while the mean of likelihood of the Bayesian analysis was –LnL = 6589.51.

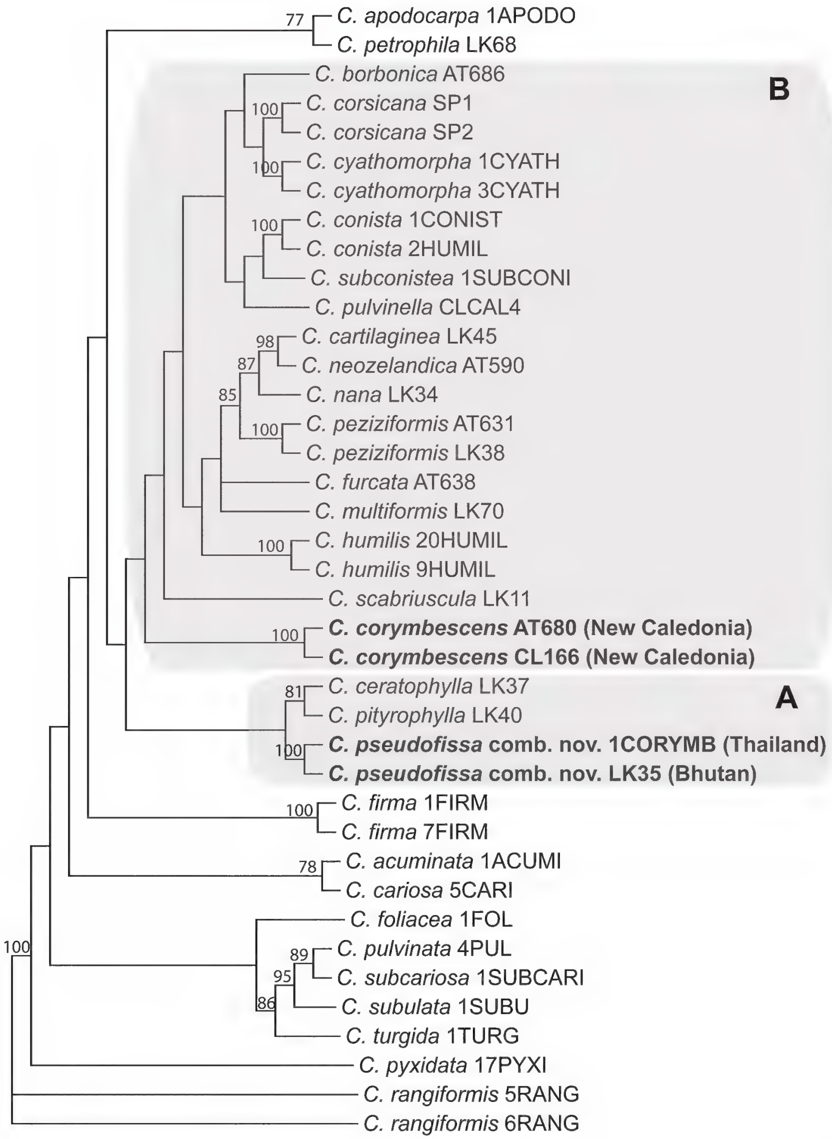

Fig. 1. *Cladonia* spp. phylogeny: consensus tree of the two most parsimonious trees based on the concatenated matrix of ITS rDNA and RPB2. The numbers on the branches represent bootstrap values (>75%).

In all the analyses the specimens from the Himalayan region and the specimens from New Caledonia formed two clades; however, the relationships

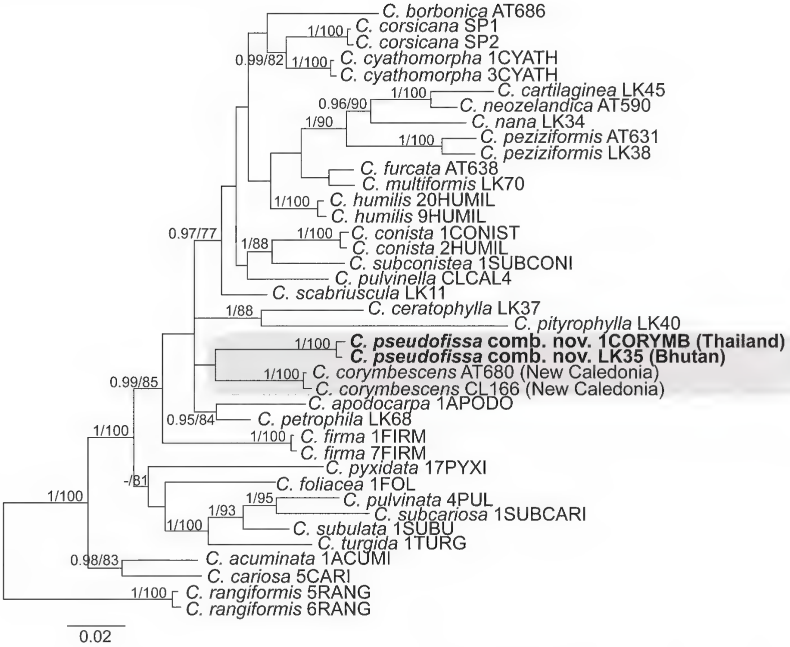

Fig. 2. *Cladonia* spp. phylogeny: 50% consensus majority tree of Bayesian analysis based on the concatenated matrix of ITS rDNA and RPB2. Posterior probability of bayesian analysis (>0.95) and bootstrap values of ML analyses (>75%) are shown on the branches.

were not the same in all the analyses. In the MP analysis (Fig. 1) the specimens from the Himalayan region (in the tree *C. pseudofissa*) are closely related with *C. pityrophylla* Nyl. and *C. ceratophylla* (Sw.) Spreng. (Fig. 1, clade A), but this relationship was not well supported (bootstrap <70%). The specimens from New Caledonia cluster in a clade with specimens representing the *C. furcata* group, *C. humilis* group, *C. corsicana* (Rondon & Vězda) Pino-Bodas et al., *C. borbonica* Nyl., *C. cartilaginea* Müll. Arg., and *C. neozelandica* Vain. (Fig. 1, clade B). The ML and Bayesian analyses generated trees with the same topology, and only the 50% consensus majority-rule tree from Bayesian analyses is shown (Fig. 2). The specimens from New Caledonia (*C. corymbescens*) and the specimens from the Himalayan region (*C. pseudofissa*) formed two clades that are closely related, although the relationship was not supported in any of the analyses.

Other differences between the MP tree and the Bayesian tree are that *C. pyxidata* (L.) Hoffm. was shown as basal in the MP analysis, while the Bayesian analysis showed *C. cariosa* (Ach.) Spreng. and *C. acuminata* (Ach.) Norrl. in the basal clade. Additionally, the clade of *C. conista* (Nyl.) Robbins, *C. subconistea* Asahina, and *C. pulvinella* S. Hammer is closely related to a clade of *C. cyathomorpha* Stirt. ex Walt. Watson, *C. corsicana*, and *C. borbonica* in the MP analysis, but not in the Bayesian analysis.

Taxonomy

Cladonia corymbescens Nyl. ex Leight., Ann. Mag. Nat. Hist., ser. 3, 18: 407. 1866. FIG. 3A

TYPE (Abbayes 1974: 112): New Caledonia, Mont de M'bée, 1855–1860 E. Vieillard 1785 (PC, lectotype, G, H-NYL 38414, PC-Lenormand, isolectotypes). [The lectotypification by Abbayes was overlooked by Ahti in Stenroos (1988: 126), Ahti (1993) and other recent authors, but the type collection cited is the same].

= *Cladonia novoguineensis* Zahlbr., Bot. Jahrb. Syst. 62: 455. 1929.

TYPE: Papua New Guinea ["Nordöstliches Neu-Guinea, Kaiser Wilhelmsland"], Morobe Prov., Sarawaged Mts. ["Saruwaged-Gebirg"], 3600–4000 m, C. Keysser 66 ["68"] (W, holotype).

Primary thallus 0.5–1 × 1.5–2 mm, soon disappearing. Podetia (2–)3–7 cm tall, 0.7–1.5 mm thick, pale to dark brown, medulla hardly melanotic at base, erect, dichotomously, trichotomously or irregularly richly branched, axils open, at least some podetia stout, sterile podetia have thin, subulate tips, fertile podetia becoming thicker, forming characteristic lateral fissures and perforations, branches often flattened. Surface highly discontinuously corticated, especially towards the base, in part sparsely microsquamulose or with larger squamules, sometimes verruculose, apical parts hardly pruinose. Podetial wall 120–220 µm thick, with indistinct cortex 10–20 µm, medulla 150–200 µm, and stereome 50–60 µm, hard. Pycnidia terminal or sometimes lateral on podetia, rarely on basal squamules, containing hyaline slime, 1.5 mm in diam, conidia 6–7 × 1 µm. Apothecia 0.8–1 mm wide, terminal, on every tip on fertile podetia, sometimes deformed, brown, spores not observed.

CHEMISTRY—K+ yellow, PD+ red, rarely PD–. CHEMOTYPE 1: Atranorin (major), fumarprotocetraric acid (major), protocetraric acid (minor), confumarprotocetraric acid (minor), rarely rangiformic and norrangiformic acids, occasionally 1–4 unknown minor substances and 1–3 terpenoids. CHEMOTYPE 2: Atranorin only, the fumarprotocetraric acid complex lacking (then PD–) (Stenroos 1988). If chemotype 2 proves to represent a distinct taxon, the name *Cladonia novoguineensis* is applicable for that (no fresh material for DNA analysis was available).

HABITAT—Terrestrial in bogs, grasslands, road banks, rarely on tree stumps, at 1500–4400 m in Papua New Guinea (Stenroos 1988: 126).

DISTRIBUTION—Australia (New South Wales, Queensland), Indonesia (Java), New Caledonia, New Zealand (North Island), Papua New Guinea, Philippines (Luzon).

ADDITIONAL SPECIMENS EXAMINED (See also Stenroos 1988) — **NEW CALEDONIA**, between Yaté and Goro, 1966, Hill 11741 (BM, H). **PAPUA NEW GUINEA**, **EASTERN HIGHLANDS**, Mt. Wilhelm, Pindaunde Lake, 3540 m, 1965, A. C. Jermy 5327 (BM, H; richly fertile!); **MOROBE PROV.**, Mt. Sarawaket Southern Range, 4 km NEE of Lake Gwam, 2850 m, 1981, T. Koponen 31662 (H, LAE); **SOUTHERN HIGHLANDS**, Mt. Giluwe, 3500 m, 1982, H. Streimann 24151 (CANB, H, LAE; chemotype 2). **PHILIPPINES**, **LUZON**, Mountain Prov., Alab on road Baguio to Bontoc, 850 m, 1976, W. S. Gruezo 2030 (CAHP, H).

Cladonia pseudofissa (Asahina) Ahti, Pino-Bodas & S. Stenroos, **stat. et comb. nov.** FIG. 3B

MYCOBANK MB 809612

≡ *Cladonia rangiformis* var. *pseudofissa* Asahina, Fl. E. Himalaya: 595. 1966.

TYPE: India, Sikkim, Jongri, 4000 m, 1960, M. Togashi 157 (TNS, holotype).

= *Cladonia erythrosperma* var. *thomsonii* Vain., Acta Soc. Fauna Fl. Fenn. 4: 376. 1887, syn. nov. [non *Cladonia thomsonii* Ahti].

TYPE: India, "India orientalis", [1847–51], J. D. Hooker & T. Thomson 2145 (PC, holotype).

= *Cladonia rangiformis* var. *incurva* Müll. Arg., Flora 74: 72. 1891, syn. nov. [non *Cladonia incurva* Ahti].

TYPE: India, Himachal Pradesh (?), Damodar ["Damdar"] Valley, 4200 m, J. F. Duthie (BM, holotype).

The earliest names for *C. pseudofissa* are *C. erythrosperma* var. *thomsonii* and *C. rangiformis* var. *incurva*, but they cannot be adopted at species level because these epithets are already occupied in *Cladonia* by *C. thomsonii* Ahti (1978) and *C. incurva* Ahti (1961).

Primary thallus rarely visible, soon disappearing, consisting of minute squamules. Podetia 1–4(–5) cm tall, to 1 mm thick, almost white, ashy grey or more rarely dark brown, medulla clearly melanotic at base; often very delicate, ascendant to erect, usually somewhat curly in appearance, without any stouter sterile podetia, repeatedly subcorymbosely dichotomously branched, apices subulate, ascyphose, axils usually perforated. Fertile podetia somewhat thicker. Surface very matt, minutely fibrous or pruinose, often very smooth and almost continuously corticate, with a few very small squamules, longitudinally somewhat fissured, esorediate. Podetial wall 150–160 μm thick, with distinct cortex 10–15 μm, medulla 75–100, stereome 25–50 μm thick, softish rather than cartilaginous. Pycnidia always at tips of podetia, shortly stalked, pyriform, black, c. 2 × 1 mm; conidia or colour of slime not observed. Apothecia dark brown, 0.5–1 mm, subglobose, on tips of flattened branchlets, spores not observed.

CHEMISTRY—K+ yellow, PD+ orange red. Contains atranorin and the fumarprotocetraric acid complex.

HABITAT—On ground, often on thin soil over rocks.

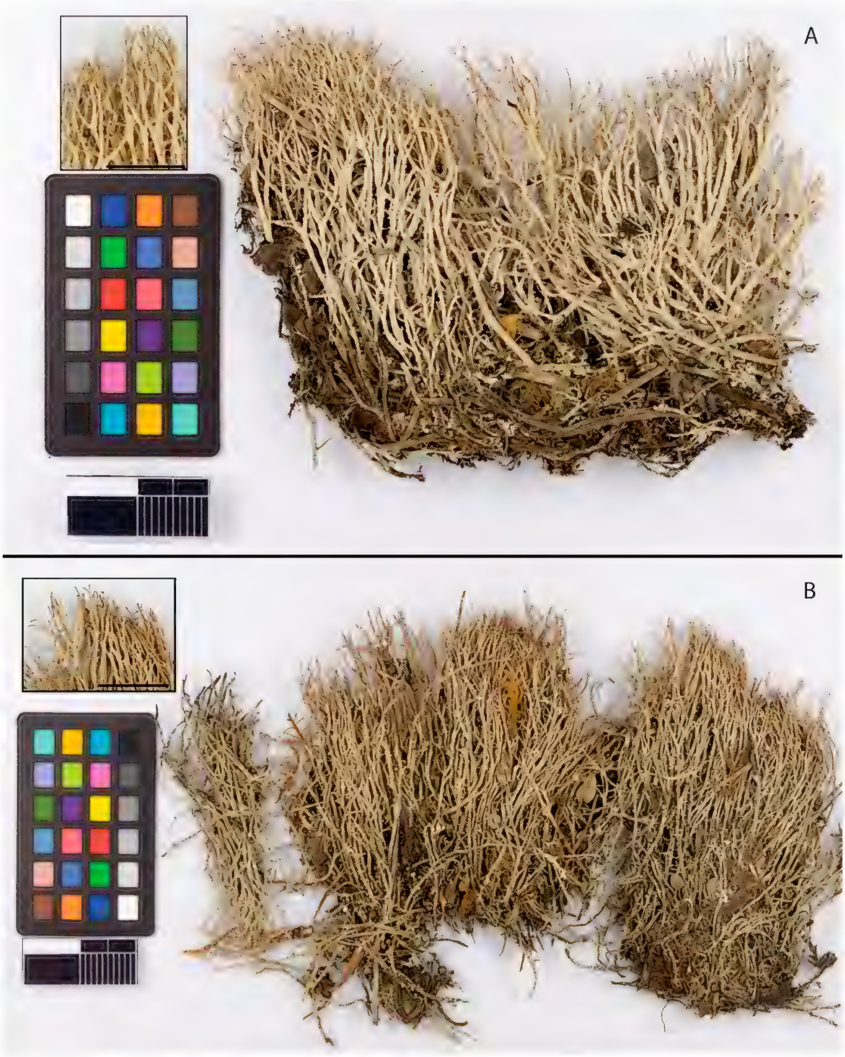

FIG. 3. A: ***Cladonia corymbescens*** (Papua New Guinea, T. Koponen 31662, H).
B: ***Cladonia pseudofissa*** (Nepal, P. Ozenda 147, H). Scale bar = 1 cm.

DISTRIBUTION—Bhutan, China (Sichuan, Tibet, Yunnan), India (Arunachal Pradesh, Himachal Pradesh, Kerala, Sikkim, Tamil Nadu, Uttarkhand, West Bengal), Malaysia (Sabah), Nepal, Thailand.

ADDITIONAL SPECIMENS EXAMINED — BHUTAN, PARO DISTRICT: below Jaley la Dzong, 2500-3000 m, 1998, U. Søchting 9206 (C, H; DNA voucher, Stenroos et al. (2002)

and GenBank, as *C. corymbescens*). **CHINA, Sichuan**: Miyi Co., 3200 m, 1983, L. S. Wang 83-834 (H, KUN). **Tibet**: Kang Ding Co., 1976, Z. Mu 5304 (H, KUN); **Yunnan**: Lijiang Co., 1985, L. S. Wang 85-140 (H, KUN). **INDIA, Sikkim**: North Sikkim District, Phune–Yakche, 3000 m, 1996, G. P. Sinha 1103 (ASSAM, H). **Uttarakhand**: Pithoragarh District, Satgarh–Dhawj, 2700 m, 1989, D. K. Upreti L-18438 (H, LWG). [For details of further Indian collections, see Rai et al. 2014, as *C. corymbescens.*] **NEPAL, Farwestern Region**: Seti, Dadachaur, Nhuna Khola, 2700 m, 1973, P. Ozenda 147C (G, H).

Discussion

Cladonia pseudofissa was included by Abbayes (1974) in *C. corymbescens.* He also thought that *C. perfossa* Nuno, described from Taiwan, belongs here. However, as discussed by Ahti & Lai (1979) and Stenroos (1988: 126), *C. perfossa* is probably a distinct species (podetia short and thick; a topotype re-examined in H), although still very poorly known.

Asahina (1966) clearly included the present species in *C. rangiformis* because he found that it contains atranorin in addition to fumarprotocetraric acid; such a strain is known in *C. rangiformis*. In habit *C. pseudofissa* does resemble *C. rangiformis* to some extent, but *C. rangiformis* is not known to range east of northwestern Iran (Litterski & Ahti 2004: 233). They are distinguished by the longitudinally split podetia and melanotic part at the base of *C. pseudofissa*.

The two segregated species generally differ in habit and colour, *C. corymbescens* being taller, erect, brownish (much resembling *C. furcata* (Huds.) Schrad.) while *C. pseudofissa* is smaller, thinner, not readily erect, and with an ashy grey or whitish tint. In addition, *C. corymbescens* is hardly blackening (melanotic) at base, whereas *C. pseudofissa* is clearly blackening.

The phylogenetic analyses by Stenroos et al. (2002) demonstrated that *C. corymbescens* was not closely related to the species of the *C. furcata* group (*C. farinacea* (Vain.) A. Evans, *C. furcata*, *C. multiformis* G. Merr., and *C. scabriuscula* (Delise) Nyl.); it seemed to be related to *C. petrophila* R.C. Harris and *C. apodocarpa* Robbins instead. The phylogenetic study of *Cladonia* in Thailand by Parnmen et al. (2008) grouped one specimen corresponding to *C. pseudofissa* with *C. rudis* Ahti & Parnmen. Morphologically this species is closely related to the species of *C. furcata* (Ahti et al. 2008). Our present phylogenetic analyses do not allow conclusions on the phylogenetic relationships of *C. corymbescens* and *C. pseudofissa*. Until further *Cladonia* species are included in the phylogenetic analyses, the placement of these species will remain uncertain. The ML and Bayesian analyses suggest that they might be related, but this relationship is not supported, and the branches in both analyses are very long. The MP analysis groups *Cladonia pseudofissa* with *C. ceratophylla* and *C. pityrophylla*, but this relationship is not well supported either. A denser sampling of taxa is needed to clarify the phylogenetic relationships between the species.

As far as is known, the geographic ranges of *C. corymbescens* and *C. pseudofissa* do not overlap; *C. corymbescens* is primarily Australasian to Melanesian and *C. pseudofissa* is Himalayan in distribution. However, the *Cladonia* flora of Indonesia is very poorly known.

Various data support *C. pseudofissa* as a species distinct from *C. corymbescens*. The phylogenetical analyses based on two independent loci separate *Cladonia pseudofissa* and *C. corymbescens* into two well-supported clades, clades that are also supported in the single gene analyses (data not shown). According to the genealogical concordance in phylogenetic species recognition (Taylor et al. 2000), these clades represent different species. In addition, morphological and distributional differences confirm that they are two different taxa.

Acknowledgments

We are grateful for fresh material of *Cladonia* collected by Dr. Maarten Christenhusz (London) in New Caledonia. We are also grateful to Dr. Philippe Clerc (Geneva), who sent our herbarium (H) a large collection of *Cladonia* from the Himalayas. Dr. Jaana Haapala helped us in photographing. Our sincere thanks to Dr. A. R. Burgaz and Dr. M. Piercey-Normore for their valuable comments to improve the manuscript.

Literature cited

Abbayes H des. 1974. *Cladonia* du Népal. Lichenes, *Cladoniaceae* (Flechten des Himalaya 9). Khumbu Himal 6(2): 111–116. Innsbruck, München: Universitätsverlag Wagner.

Ahti T. 1961. Taxonomic studies on reindeer lichens (*Cladonia*, subgenus *Cladina*). Ann. Bot. Soc. Zool. Bot. Fenn. 'Vanamo' 32(1): 1–160.

Ahti T. 1978. Two new species of *Cladonia* from western North America. Bryologist 81: 334–338.

Ahti T. 1993. Names in current use in the *Cladoniaceae* (lichen-forming ascomycetes) in the ranks of genus to variety. Regnum Vegetabile 78: 59–106.

Ahti T, Lai MJ. 1979. The lichen genera *Cladonia*, *Cladina* and *Cladia* in Taiwan. Ann. Bot. Fenn. 16: 228–236.

Ahti T, Parnmen S, Mongkolsuk P. 2008. Three new species of *Cladonia* from Thailand. Sauteria 15: 15–19.

Argüello A, Del Prado R, Cubas P, Crespo A. 2007. *Parmelia quercina* (*Parmeliaceae*, *Lecanorales*) includes four phylogenetically supported morphospecies. Biol. J. Linn. Soc. 91: 455–467. http://dx.doi.org/10.1111/j.1095-8312.2007.00810.x

Asahina Y. 1966. Lichens. 592–610, in: H Hara (ed.). The flora of Eastern Himalaya. University of Tokyo.

Awasthi DD, Ahti T. 2007. *Cladonia* P. Browne (*Cladoniaceae*). 79–116, in: DD Awasthi (ed.). A compendium of the macrolichens from India, Nepal and Sri Lanka. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Fontaine KM, Ahti T, Piercey-Normore MD. 2010. Convergent evolution in *Cladonia gracilis* and allies. Lichenologist 42: 323–338. http://dx.doi.org/10.1017/S0024282909990569

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes. Application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Kelly LJ, Hollingsworth PM, Coppins BJ. 2011. DNA barcoding of lichenized fungi demonstrated high identification success in a floristic context. New Phytol. 191: 288–300. http://dx.doi.org/10.1111/j.1469-8137.2011.03677.x

Kotelko R, Piercey–Normore MD. 2010. *Cladonia pyxidata* and *C. pocillum*; genetic evidence to regard them as conspecific. Mycologia 102: 534–545. http://dx.doi.org/10.3852/09-030

Litterski B, Ahti T. 2004. World distribution of selected European *Cladonia* species. Symb. Bot. Ups. 34(1): 205–236.

Lutzoni F, Kauff F, Cox C, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. Am. J. Bot. 91: 1446–1480. http://dx.doi.org/10.3732/ajb.91.10.1446

Muggia L, Pérez-Ortega S, Fryday A, Spribille T, Grube M. 2014. Global assessment of genetic variation and phenotypic plasticity in the lichen-forming species *Tephromela atra*. Fungal Diversity 64: 233–251. http://dx.doi.org/10.1007/s13225-013-0271-4

Núñez-Zapata J, Divakar PK, Del-Prado R, Cubas P, Hawksworth DL, Crespo A. 2010. Conundrums in species concepts: the discovery of a new cryptic species segregated from *Parmelina tiliacea* (*Ascomycota*: *Parmeliaceae*). Lichenologist 43: 603–616. http://dx.doi.org/10.1017/S002428291100051X

Nylander JAA. 2004. MrModelTest 2.1. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Nylander JAA, Wilgenbusch DL, Warren, JC, Swofford DL. 2008. AWTY (Are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24: 581–583. http://dx.doi.org/10.1093/bioinformatics/btm388

Orange A, James PW, White FJ. 2001. Microchemical methods for the identification of lichens. British Lichen Society, London.

Parnmen S, Rangsiruji A, Mongkolsuk P, Ahti T. 2008. Phylogenetics of lichens in the genus *Cladonia* (Cladoniaceae) in northern and northeastern Thailand. Sauteria 15: 385–402.

Parnmen S, Leavitt SD, Rangsiruji A, Lumbsch HT. 2013. Identification of species in the *Cladia aggregata* group using DNA barcoding (*Ascomycota*: *Lecanorales*). Phytotaxa 115: 1–14. http://dx.doi.org/10.11646/phytotaxa.115.1.1

Pino-Bodas R, Burgaz AR, Martín MP. 2010a. Elucidating the taxonomic rank of *Cladonia subulata* versus *C. rei* (*Cladoniaceae*). Mycotaxon 113: 311–326. http://dx.doi.org/10.5248/113.311

Pino-Bodas R, Martín MP, Burgaz AR. 2010b. Insight into the *Cladonia convoluta–C. foliacea* (*Cladoniaceae*, *Ascomycota*) complex and related species, revealed through morphological, biochemical and phylogenetic analyses. Syst. Biodivers. 8: 575–586. http://dx.doi.org/10.1080/14772000.2010.532834

Pino-Bodas R, Burgaz AR, Martín MP, Lumbsch HT. 2011. Phenotypical plasticity and homoplasy complicate species delimitation in the *Cladonia gracilis* group (*Cladoniaceae*, *Ascomycota*). Org. Divers. Evol. 11: 343–355. http://dx.doi.org/10.1007/s13127-011-0062-2

Pino-Bodas R, Burgaz AR, Martín MP, Lumbsch HT. 2012a. Species delimitations in the *Cladonia cariosa* group (*Cladoniaceae*, *Ascomycota*). Lichenologist 44: 121–135. http://dx.doi.org/10.1017/S002428291100065X

Pino-Bodas R, Ahti T, Stenroos S, Martín MP, Burgaz AR. 2012b. *Cladonia conista* and *C. humilis* (*Cladoniaceae*) are different species. Bibl. Lichenol. 108: 161–176. http://dx.doi.org/1436-1698/2012/108-161

Pino-Bodas R, Martín MP, Burgaz AR. 2012c. *Cladonia subturgida* and *C. iberica* (*Cladoniaceae*) form a single, morphologically and chemically polymorphic species. Mycol. Prog. 11: 269–278. http://dx.doi.org/10.1007/s11557-011-0746-1

Pino-Bodas R, Ahti T, Stenroos S, Martín MP, Burgaz AR. 2013a. Multilocus approach to species recognition in the *Cladonia humilis* complex (*Cladoniaceae*, *Ascomycota*). Am. J. Bot. 100: 664–678. http://dx.doi.org/10.3732/ajb.1200162

Pino-Bodas R, Martín MP, Burgaz AR, Lumbsch HT. 2013b. Species delimitation in *Cladonia* (*Ascomycota*): a challenge to the DNA barcoding philosophy. Mol. Ecol. Resour. 13: 1058–1068. http://dx.doi.org/10.1111/1755-0998.12086

Rai H, Khare R, Upreti DK, Ahti T. 2014. Terricolous lichens of India: taxonomic keys and description. 17–294, in: H Rai, DK Upreti (eds). Terricolous lichens in India 2. Springer, Heidelberg. http://dx.doi.org/10.1007/978-1-4939-0360-3_2

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque A, Chen V, Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode for Fungi. Proc. Natl. Acad. Sci. USA 109: 6241–6246. http://dx.doi.org/10.1073/ pnas.1117018109.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. http://dx.doi.org/10.1093/bioinformatics/btl446

Steinová J, Stenroos S, Grube M, Škaloud P. 2013. Genetic diversity and species delimitation of the zeorin-containing red-fruited *Cladonia* species (lichenized *Ascomycota*) assessed with ITS rDNA and β-tubulin data. Lichenologist 45: 665–684. http://dx.doi.org/10.1017/S0024282913000297

Stenroos S. 1988. The family *Cladoniaceae* in Melanesia. 3. *Cladonia* sections *Helopodium*, *Perviae* and *Cladonia*. Ann. Bot. Fenn. 25: 117–148.

Stenroos S, Hyvönen J, Myllys, L, Thell, A, Ahti T. 2002. Phylogeny of the genus *Cladonia* s. lat. (*Cladoniaceae*, *Ascomycetes*) inferred from molecular, morphological, and chemical data. Cladistics 18: 237–278. http://dx.doi.org/10.1006/clad.2002.0202

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. 2000. Species recognition and species concepts in fungi. Fungal Genet. Biol. 29: 21–32. http://dx.doi.org/10.1006/fgbi.2000.1228

Vainio EA. 1887. Monographia Cladoniarum universalis 1. Acta Soc. Fauna et Flora Fenn. 4: 1–509.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR protocols: a guide to methods and applications. Academic Press, Orlando FL.

Yahr R, Vilgalys R, DePriest PT. 2006. Geographic variation in algal partners of *Cladonia subtenuis* (*Cladoniaceae*) highlights the dynamic nature of a lichen symbiosis. New Phytol. 171: 847–860. http://dx.doi.org/10.1111/j.1469-8137.2006.01792.x